Immunogenetic and clinical aspects of autoimmune hepatitis

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CHAPTER 6

The assessment of the histopathological key-features in autoimmune hepatitis

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ABSTRACT

Aim

In this study we aimed to evaluate the use of typical histological features of both the revised original (1999) and simplified (2008) criteria in the diagnosis of autoimmune hepatitis (AIH) in clinical practice.

Methods

We performed a detailed histopathological evaluation of the pre-treatment biopsies 63 AIH patients and used biopsies of 62 untreated chronic viral hepatitis patients [hepatitis B (n=21) or C (n=41)] as a reference cohort. Biopsies were systematically reviewed for inflammation, fibrosis and the presence of interface hepatitis, plasma cells, rosettes and emperipolesis using a well-defined assessment method.

Results

AIH biopsies displayed more interface hepatitis (87% versus 63%, p=0.002), more plasma cell rich infiltrates (48% versus 27%, p=0.02), rosettes (49% versus 23%, p=0.004) and emperipolesis (78% versus 50%, p=0.001) than chronic viral hepatitis biopsies. Emperipolesis (p=0.01) and rosettes (p<0.01) were superior to plasma cells and interface hepatitis as independent predictors for AIH. Moderate to severe lymphocytic cholangitis was found in 28% of AIH patients.

Conclusion

Emperipolesis and rosette formation are superior histological predictors of AIH when compared to the classical hallmark features of interface hepatitis and plasma cells. In addition, moderate to severe lymphocytic cholangitis does not preclude the diagnosis of AIH.

INTRODUCTION

Autoimmune hepatitis (AIH) is generally a progressive, chronic hepatitis of unknown aetiology. In the absence of a golden standard, the diagnosis of AIH is based on a combination of clinical, biochemical and histological findings. In addition, hyperimmunoglobulinemia (IgG), autoantibodies [Type-1: smooth muscle antibodies (SMA), antinuclear antibodies (ANA) and/or soluble liver/liver-pancreas antibodies (SL/LPA); Type-2: liver-kidney microsomal-1 antibodies (LKM-1)] can be found in the serum.[1] In order to standardize the diagnostic process, the International Autoimmune Hepatitis Group (IAIHG) devised a diagnostic scoring system which was revised in 1999.[2, 3] In 2008 the same expert group published a more comprehensive set of diagnostic criteria to aid diagnosing AIH in daily practice. In both sets of criteria histological proof of (interface) hepatitis is mandatory to make a definite diagnosis of AIH [1999 criteria ≥15 points (0-24); 2008 criteria: ≥7 points (0-8)],[3, 4] yet they differ with respect to other histological features. Thus in the 1999 criteria interface hepatitis (+3 points), presence of plasma cells (+1 point) and rosettes (+1 point) are regarded as typical histological features of AIH.[2, 3] The simplified criteria state that portal lymphocytic/lymphoplasmacytic infiltrates extending into the lobules, emperipolesis and hepatic rosette formation are typical features.[4] Although the presence of these features thus contributes to the diagnostic score of AIH, the 1999 and 2008 criteria do not provide a descriptive definition or cut-off value to assess emperipolesis and rosettes. As such, these features may prove difficult to assess in clinical practice and they have not been specifically validated in confirmation studies.[5-7] Furthermore, biliary changes, including lymphocytic cholangitis, are regarded to be 'adverse' findings and make a definite diagnosis of AIH less likely in both sets of criteria,[2, 4] despite being present in up to 40-60% of AIH patients in previous reports.[2, 8, 9] The dissimilarities between the scoring systems, as well as the lack of clear-cut definitions of the typical AIH features, may impair the adequate and timely recognition and the treatment of AIH.[10] The aim of the present study was to ascertain the clinical value of these features using a practical assessment method. We compared the frequencies of these criteria in liver biopsies of AIH patients with a reference group of chronic viral hepatitis patients.

MATERIALS AND METHODS

Patients

We reviewed the pre-treatment biopsies of patients with a clinical diagnosis of AIH and a diagnostic revised IAIHG-score (1999) of at least 10 or higher (a probable or definite AIH diagnosis) seen at the out-patient clinic of the VU University Medical Center (tertiary

referral centre) between 1990 and 2012. Diagnostic IAIHG scores were calculated from retrospectively collected clinical, biochemical and serological data and the original histopathological assessment of the biopsy, retrieved from patient records. In all patients clinical and biochemical parameters were assessed to exclude other aetiologies such as alcohol, drugs and metabolic disorders. Viral hepatitis was excluded by serological testing. We excluded patients with evidence for the presence of clinical overlap syndromes with primary biliary cirrhosis (PBC) or primary sclerosing cholangitis (PSC) in case of positive anti-mitochondrial antibodies (AMA) or distinct cholangiographic abnormalities, respectively. In 21 of 92 eligible AIH patients we were unable to retrieve biopsies, since a pre-treatment biopsy had not been performed (n=9) or was not available from the referring hospital where it had been performed (n=12). We excluded eight AIH biopsies from analysis after revision, because they contained less than four portal areas. Finally, 63 diagnostic biopsies of untreated AIH patients (76% women) with a mean age of 46 years at diagnosis [standard deviation (SD): ±18.0] were included. The results of the AIH biopsies were compared for reference with 67 available biopsies of untreated chronic viral hepatitis patients (≥ 6 months), seen at the out-patient clinic of the VU University Medical Center between 2004 and 2012. Five biopsies of chronic viral hepatitis patients had less than four portal areas and were excluded from the analysis. Sixty-two controls (21 Hepatitis B patients and 41 Hepatitis C patients; 35% women) with a mean age of 47 years (SD: ±11.4) were included for analysis. This study was performed according to the institutional ethical guidelines set by the institutional review board and in line with the declaration of Helsinki and the Dutch legislation on use of left-over pathology material.[11]

Biopsies

Liver biopsies had been obtained with a standard fourteen gauge needle under ultrasound guidance by the treating physician or radiologist. Following fixation in 4% paraformaldehyde, haematoxylin & eosin (H&E), reticulin and collagen (Sirius red, Elastica van Gieson) stain and immunohistochemistry for cytokeratin-7 (CK-7) had routinely been performed. Since part of biopsies was retrieved from referring centers, the reticulin, collagen and CK-7 stains were not always available. The biopsies were reviewed and scored simultaneously by an experienced hepatopathologist (E.B.) and a committed hepatopathology investigator (Y.B.), who were blinded to clinical patient data. Portal-periportal and lobular inflammation activity grade (ranges: 0-4) as well as fibrosis stage (range: 0-4) were scored according to Scheuer (Table 1).[12] Interface hepatitis was defined as a portal-periportal Scheuer inflammation score of ≥2 (0 = none, 1 = portal inflammation, 2 = focal interface activity, 3 = interface activity in 33-66% portal areas, 4 = interface activity > 66% portal areas). In case of bridging necrosis (lobular activity score 4, Table 1), the staging of fibrosis in liver biopsies was not performed. Infiltrate analysis was performed on H&E staining. The presence of neutrophils, eosinophils, lymphocytes and plasma

cells in the portal and lobular infiltrates was assessed on a semi-quantitative scale (0 = none, 1 = <20% of inflammatory cells, 2 = 20-50% of inflammatory cells, 3 = >50% of inflammatory cells) (Table 1, Figure 1). Hepatic rosettes were defined as configurations

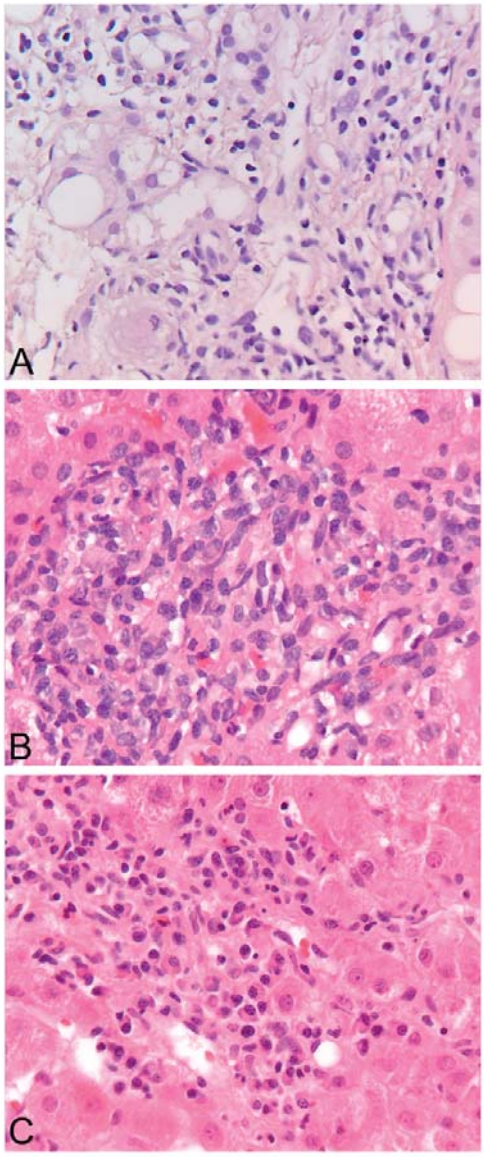

Fig. 1. Plasma cells. Three representative examples of the semi-quantitative score for the presence of plasma cells. A. < 20%, B 20-50%, C > 50%

of hepatocytes arranged around a central lumen. We applied a semi quantitative score (0 = none, 1 = 5 - 25% of the parenchyma affected, 2 = 25-50% of the parenchyma affected, 3 = >50 % of the parenchyma affected) to reliably ascertain the presence and the extent of rosettes formation in the available reticulin stains (Figure 2). To assess the presence of emperipolesis we used a simple strategy. Biopsies were scored positive, if the H&E stains showed evidence of at least one complete inclusion of a mononucleated inflammatory cell with visible cytoplasm (lymphocyte/plasma cell) into a hepatocyte (Figure 3A-B). Inclusions of nuclear fragments with chromatin condensation (pyknosis)

were not considered emperipolesis (Figure 3C-D). Biliary inflammation was assessed on a semi-quantitative scale (0 = none, 1 = mild infiltration, 2 = moderate lymphocytic cholangitis, 3 = severe lymphocytic cholangitis with destruction of the bile duct, Figure 4). Ductopenia was defined as the absence of bile ducts in more than 50% of portal areas. Ductular reaction and biliary metaplasia as a sign of cholate stasis were only assessed if CK-7 stains were available. In addition, the presence of lymphocytic endophlebitis and parenchymal granulomas were assessed. Steatosis was scored on a semi quantitative scale (0 = none, 1 = 5 - 33% of the parenchyma affected, 2 = 33-66% of the parenchyma affected, 3 = >66 % of the parenchyma affected).

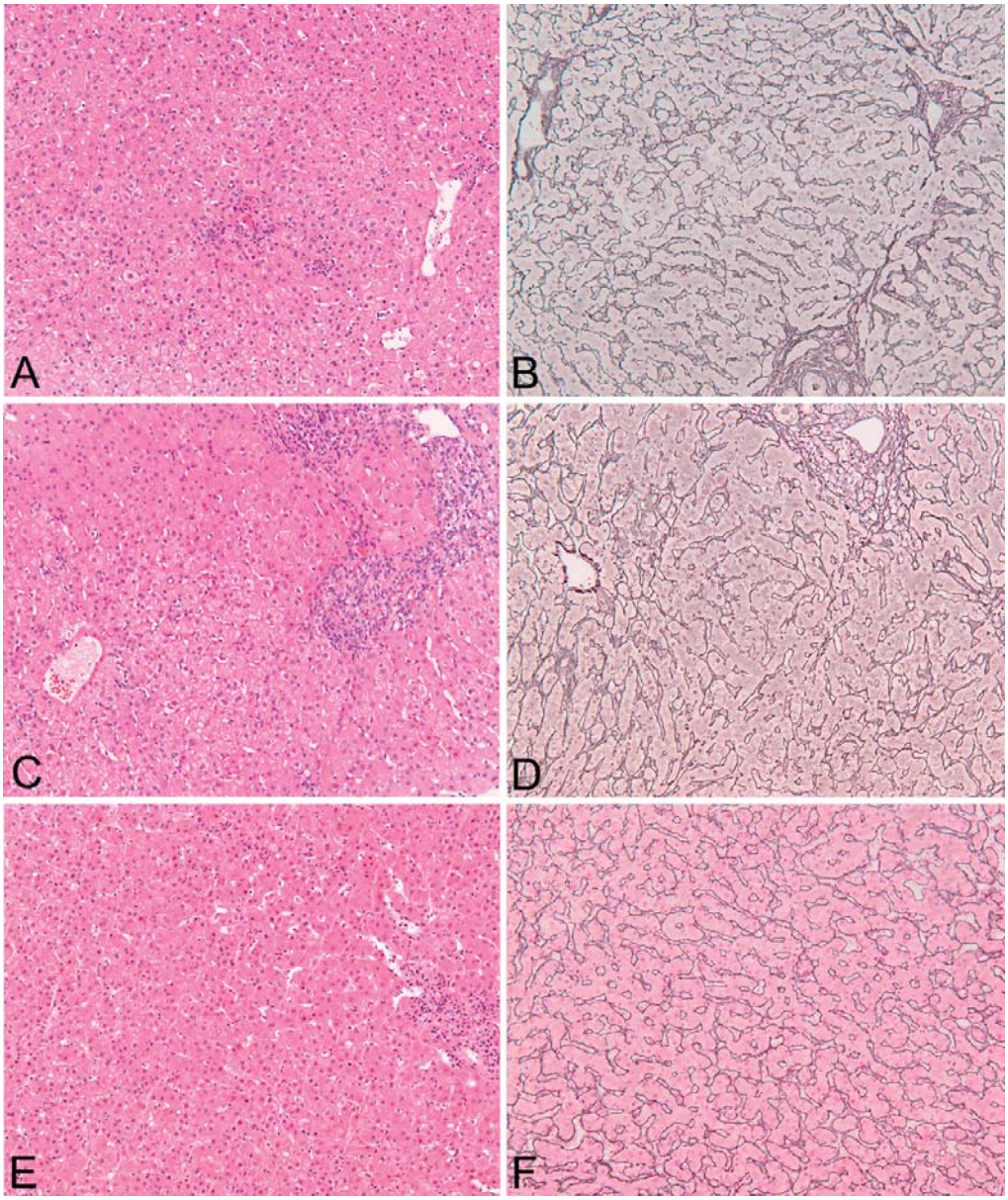

Fig. 2. Hepatic rosettes. Representative examples of the semi-quantitative score for the presence of rosettes. Both H&E (left) and reticulin (right) stains of the same biopsy are shown for each score. A and B, score 1 (5 - 25% of the parenchyma affected); C and D, score 2 (25-50% of the parenchyma affected); E and F score 3 (>50 % of the parenchyma affected).

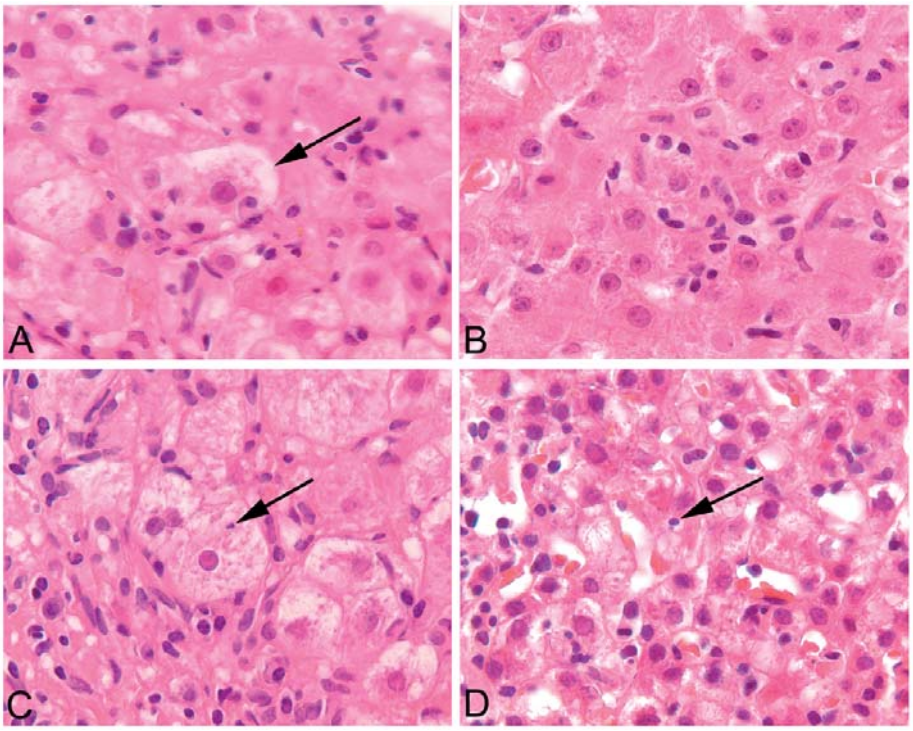

Fig. 3. Emperipolesis. Examples of emperipolesis. A and B show emperipolesis of plasmacells and lymphocytes. C and D show hepatocytes with engulfed apoptotic cell fragments.

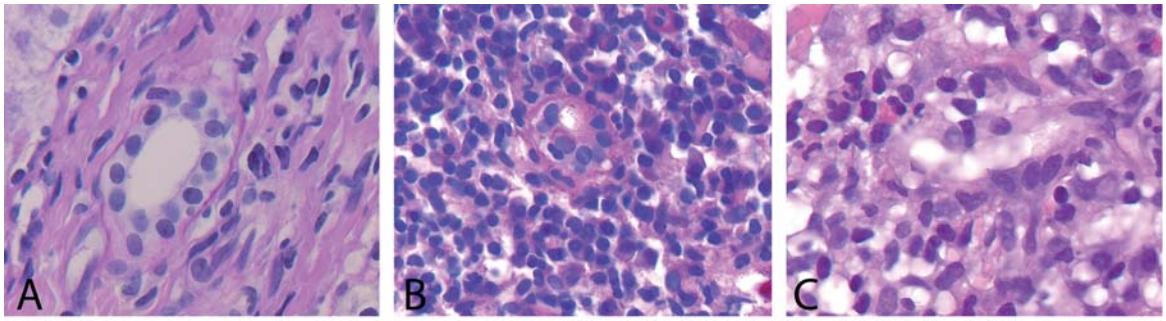

Fig. 4. Biliary inflammation. Examples of the semi-quantitative score of biliary inflammation. A, score 1 (mild infiltration); B, score 2 (lymphocytic cholangitis); C, score 3 (extensive lymphocytic cholangitis with destruction of the bile duct).

Statistical analysis

Statistical analysis was performed using IBM SPSS 20.0 (IBM Corp., Armonk, NY). Demographic and therapy specific data are given descriptively in mean and standard deviation (SD) and median and interquartile range (IQR) in case of Gaussian distribution and non-Gaussian distribution respectively. Statistical testing of continuous variables between groups was done with Student-*t* test (Gaussian) and the Mann-Whitney-*U* test or Wilcoxon signed rank test (non-Gaussian). Proportional differences were tested for statistical significance with the χ^2-test or Fisher's exact test. A p-value of <0.05 was considered statistically significant. The presence of interface hepatitis, plasma cells and

rosettes were positive at a cut-off value of ≥2. Sensitivity and specificity for each typical AIH feature were calculated to evaluate the individual discriminative value in chronic hepatitis biopsies. We performed univariate logistic regression on the typical histological features (interface hepatitis, rosettes, plasma cells and emperipolesis) in patients in which all histological features had been assessed. In the regression analysis, a p-value of <0.10 was considered statistically significant. To identify the best combination of histological predictors of AIH in this chronic hepatitis cohort, significant variables were included in a multivariate logistic regression analysis using conditional stepwise forward and backward selection.

Table 1. Scoring sheet for inflammation and fibrosis and histopathological features

Portal-periportal activity	**Grade**	**Prominent inflammatory cell types**	
None or minimal	0	Neutrophils	0/1/2/3
Portal inflammation	1	Eosinophils	0/1/2/3
Mild interfase hepatitis	2	Lymphocytes	0/1/2/3
Moderate interface hepatitis	3	Plasma cells	0/1/2/3
Severe interface hepatitis	4	**'Typical' features**	
		Emperipolesis	Yes/No
Lobular activity	**Grade**	Rosettes	0/1/2/3
None	0	**Cholestasis**	
Inflammation but no necrosis	1	Intracellular	Yes/No
Focal necrosis or acidophil bodies	2	Canalicular	Yes/No
Severe focal cell damage	3	Cholangiolar	Yes/No
Damage includes bridging necrosis	4	Cholate stasis	Yes/No
		Bile duct injury	
Fibrosis	**Stage**	Inflammation	0/1/2/3
None	0	Ductopenia	Yes/No
Enlarged fibrotic portal tracts	1	Sclerosis	Yes/No
Periportal or portal-portal septa but intact architecture	2	**Granulomas**	Yes/No
Fibrosis with architetural distortion but no cirrhosis	3	**Endophlebitis**	Yes/No
Probable or definite cirrhosis	4	**Steatosis**	0/1/2/3

Inflammation and fibrosis were assessed using the Scheuer score.[12] Other histopathological features were assessed on a semi-quantitative or binominal score.

RESULTS

Baseline characteristics

Autoimmune hepatitis patients presented with more severe biochemical disease at the time of biopsy than viral hepatitis patients, showing higher median levels of alanine

aminotransferase [ALT: 148 (IQR: 61-356) vs. 67 (IQR: 47-95) U/L, p<0.001] and aspartate aminotransferase [AST: 75 (IQR: 37-219) vs. 48 (IQR: 37-70) U/L, p=0.001]. There was also a marked difference in alkaline phosphatase (ALP) (p<0.001) and gamma-glutamyltransferase (GGT) (p<0.001) levels between AIH and viral hepatitis patients (Table 2). Autoantibody (ANA, SMA and LKM-1) results at the time of biopsy were available for 58 of the 63 (89%) AIH patients and 23 of 41 (56%) chronic hepatitis C patients (CHC) but not for chronic hepatitis B patients (Table 2). AIH patients displayed more ANA positivity than CHC patients (AIH: 55% vs. CHC: 4%, p=0.001) (Table 2). Also, AIH patients more often had SMA antibodies than CHC patients (AIH: 60% vs. CHC: 13%, p<0.001) (Table 2). Three of 48 (6%) tested AIH patients had positive LKM-1 autoantibodies.

Table 2. Baseline characteristics of autoimmune hepatitis and chronic viral hepatitis patients

	AIH		Chronic viral Hepatitis		p value
N	63		62		
Age years, Mean (SD)	46	(±18.0)	47	(±11.3)	0.8
Female, n (%)	48	(76)	21	(34)	<0.001
ALT U/L, Median (IQR)	148	(61-356)	67	(47-95)	<0.001*
AST U/L,Median (IQR)	75	(37-219)	48	(37-70)	0.001*
ALP U/L, Median (IQR)	122	(89-194)	82	(65-104)	<0.001*
GGT U/L, Median (IQR)	142	(42-348)	43	(29-98)	<0.001*
Bilirubin µmol/L, Median (IQR)	13	(8-22)	10	(7-15)	0.06*
IgG g/L, Median (IQR)	17.1	(15.1-21.9)	-	-	-
ANA ≥ 1:40, n/total (%) #	31/56	(55)	11/23	(4)	0.001
SMA ≥ 1:40, n/total (%) #	35/58	(60)	3/23	(13)	<0.001
LKM-1 ≥ 1:40, n/total (%) #	3/48	(6)	0/7	(0)	1

* = t-test after logarithmic transformation. # AIH and chronic hepatitis C patients with available auto-antibody test results. Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminostransferase; ANA, antinuclear antibodies; AST aspartate aminotransferase; GGT, gamma-glutamyltransferase; IQR, interquartile range; M, male; LKM-1, liver-kidney microsomal-1 antibodies; SMA, smooth muscle antibodies.

Inflammation and fibrosis

The biopsies of patients with AIH showed statistically significantly higher activity scores for both (peri-)portal (p=0.002) (Figure 5A) and lobular inflammation (p<0.001) (Figure 5B). The number of biopsies with bridging necrosis (lobular activity score 4) was significantly higher in AIH-biopsies (40% vs 7%, p<0.001; Figure 5B). Thirty-three biopsies were excluded from fibrosis assessment due to the presence of bridging necrosis (AIH: n=25;

viral hepatitis: n=4) or lack of appropriate collagen stains (AIH: n=4). The remaining 34 AIH and 58 viral hepatitis biopsies displayed a similar distribution of fibrosis (p=0.2), with an equal percentage of patients with histological cirrhosis (fibrosis score = 4) (12% vs 12%, p=1.0; Figure 5C).

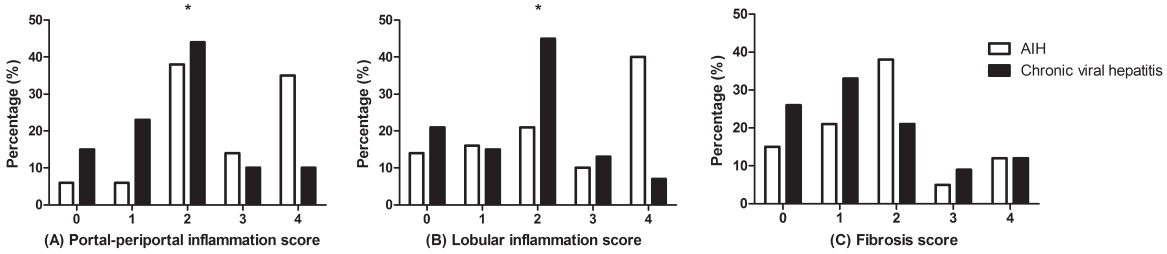

Fig. 5. Distribution of portal and lobular inflammation and fibrosis scores in AIH and chronic viral hepatitis patients. Portal-periportal (A) and lobular (B) inflammation were scored according to Scheuer et al. [12] in all biopsies. Fibrosis (C) was scored in 34 AIH and 58 chronic viral hepatitis biopsies with available collagen stains and without bridging necrosis.

Typical AIH Features

Typical histological features that contribute to the diagnosis of AIH were found more often, but not exclusively in AIH. Interface hepatitis, defined as a portal-periportal Scheuer activity score of ≥2, was present in 55 (87%) of AIH biopsies and 39 (63%) of viral hepatitis biopsies (p=0.002; Table 3). The AIH liver biopsies more often displayed plasmacellular dominated infiltrates (48% versus 27%, p=0.02) and emperipolesis (78% versus 50%, p=0.001) when compared with viral hepatitis biopsies (Table 3). In biopsies with available reticulin stains (AIH n=45; viral hepatitis n=56) rosettes were also more prevalent in AIH than in viral hepatitis (49% versus 23 %, p=0.004).

Biliary changes

Histological signs of bile duct inflammation (score ≥1) were found in 44 (70%) AIH biopsies. Moderate to severe lymphocytic cholangitis (score ≥2) was present in 29% of AIH biopsies and in 18% of viral hepatitis biopsies (p=0.2). Ductopenia and concentric periductal fibrosis were seen in 4 (6%) and 2 (3%) of AIH patients, respectively. The presence of biliary changes in AIH patients with a probable (n=16) and a definite (n=47) IAIHG score were 75% and 68% respectively (p=0.8). A recalculation of the pre-treatment 1999 IAIHG diagnostic score, without a three point deduction in case of biliary inflammation, awarded a definite AIH diagnosis (≥15 points) in 12 out of 16 (75%) patients with an initial probable AIH diagnosis (10-15 points).

Other features

Moderate to severe steatosis was more prevalent in viral hepatitis than AIH biopsies (15% vs 3%, p=0.03). In the AIH biopsies with moderate (n=1) and severe (n=1) steatosis,

no histological signs of steatohepatitis were present (no ballooning of hepatocytes). AIH biopsies displayed granulomas (11% vs 2%, p=0.06) and endophlebitis of the portal vessels (15% vs 2%, p= 0.008) more often than chronic viral hepatitis. Endophlebitis in AIH patients was more often observed in case of severe lobular and interface hepatitis with bridging necrosis (p=0.001).

Table 3. Histological features in autoimmune hepatitis and chronic viral hepatitis

	AIH	Viral Hepatitis	p value	Odds ratio	95%-confidence Interval
	n **(%)**	n **(%)**			
Histological features					
Portal-periportal activity (≥2) #	55 (87)	39 **(63)**	0.002	4.1	1.6 – 10.0
Lobular activity (=4) #	25 (40)	4 **(7%)**	<0.001	9.5	3.1-29.6
Fibrosis (=4) #^	4 (12)	7 **(12)**	1.0	1.0	0.3-3.6
Plasma cells (≥2)	30 (48)	17 **(27)**	0.02	2.4	1.1 - 5.1
Rosettes (≥2)$	23 (49)	13 **(23)**	0.004	3.4	1.5 - 8.1
Emperipolesis (≥2)	49 (78)	31 **(50)**	0.001	3.5	1.6 - 7.6
Any biliary inflammation (≥1)	44 (70)	24 **(39)**	<0.001	3.6	1.7 - 7.7
Moderate -severe cholangitis (≥2)	18 (29)	11 **(18)**	0.2	1.9	0.8 - 4.3
Steatosis (≥2)	2 (3)	9 **(15)**	0.03	0.2	0.04 - 0.9
Ductopenia	4 (6)	2 **(3)**	0.7	2.0	0.4 - 11.5
Periductal concentric fibrosis	2 (3)	0 **(0)**	0.5	-	-
Endophlebitis	9 (15)	1 **(2)**	0.008	10.6	1.3 - 86.1
Granulomas	7 (11)	1 **(2)**	0.06	7.6	0.9-63.9

Scheuer score[12]; ^ fibrosis was assessed in 34 AIH and 58 viral hepatitis biopsies with available collagen stains and without parenchymal collaps; $ Rosette formation was assessed if reticulin stains were available (AIH: n=45, viral hepatitis: n = 56).

Diagnostic accuracy of typical AIH features in chronic hepatitis

The sensitivity and specificity for the individual and combined 1999 and 2008 histological AIH criteria were calculated for the 45 AIH and 56 chronic viral hepatitis biopsies (Table 4) in which all histological features could be assessed. Both interface hepatitis and emperipolesis had high sensitivity (84% and 78%, respectively) but low specificity (36% and 50%, respectively). In contrast plasmacellular infiltration and rosettes had low sensitivity (49% and 51%, respectively), but high specificity (71% and 77%, respectively). The 1999 criteria and 2008 criteria had low sensitivity (27% and 40%, respectively) but high specificity (91% and 89% respectively) (Table 4). Univariate regression analysis on the four histological features (interface hepatitis, plasma cells, emperipolesis and rosettes) showed that each variable alone was a statistically significant predictor (Table 5). We incorporated all four histological criteria in a multivariate logistic regression model

(Table 5) and performed conditional stepwise backward and forward selection. This consistently resulted in a regression model containing both emperipolesis (OR: 3.0; 95%-CI: 1.2-7.2; p=0.02) and rosettes (OR 3.0; 95%-CI: 1.2-7.5; p=0.02) as the best discriminative predictors of AIH. In a regression analysis incorporating only the 34 cases with a definite diagnosis of AIH (IAIHG-score ≥15) and 56 chronic viral hepatitis patients, rosettes (OR: 3.6; 95%-CI: 1.4-9.5; p<0.01) and emperipolesis (OR: 4.0; 95%-CI: 1.4-11.7; p=0.01) again were the best predictors of AIH. Hence, the combination of these features was superior to plasmacellular infiltration and interface hepatitis as independent predictors for AIH in this population of chronic hepatitis patients (Table 5).

Table 4. Sensitivity and specificity of 'typical' AIH features in autoimmune hepatitis and chronic viral hepatitis

	Sensitivity	**Specificity**
	(%)	(%)
Features		
Interface hepatitis (≥2)	84	36
Plasma cells (≥2)	49	71
Rosettes (≥2)	51	77
Emperipolesis	78	50
Interface hepatitis + Rosettes + Plasma cells ("criteria 1999")	27	91
Interface hepatitis + Rosettes + Emperipolesis ("criteria 2008")	40	89
Interface hepatitis + Rosettes + Emperipolesis + Plasma cells	27	93
Rosettes + Plasma cells	27	91
Rosettes + Emperipolesis	44	88
Rosettes + Interface hepatitis	44	82

Sensitivity and specificity values were calculated in cases with all features available (autoimmune hepatitis, n=45; chronic viral hepatitis, n = 56).

Table 5. Univariate and multivariate logistic regression analysis on 'typical' histological AIH features

	Probable + definite AIH (IAIHG score ≥10)			Definite AIH (IAIHG score ≥15)		
Univariate regression	**OR**	**95%-CI**	**p value**	**OR**	**95%-CI**	**p value**
Interface hepatitis (≥2)	3.0	1.1 - 8.0	0.03	4.2	1.3 - 13.5	0.02
Plasma cells (≥2)	2.4	1.1 - 5.5	0.04	2.5	1.0 - 6.1	0.04
Rosettes (≥2)	3.5	1.5 - 8.1	<0.01	4.2	1.7 - 10.5	<0.01
Emperipolesis	3.5	1.5 - 8.4	<0.01	4.7	1.7 - 13.0	<0.01
Multivariate regression						
1999 criteria	**OR**	**95%-CI**	**p value**	**OR**	**95%-CI**	**p value**
Interface hepatitis (≥2)	2.1	0.7 - 6.1	0.2	2.8	0.8 - 10.4	0.1
Plasma cells (≥2)	1.7	0.7 - 4.2	0.3	1.6	0.6 - 4.3	0.4
Rosettes (≥2)	3.0	1.2 - 7.2	0.01	3.6	1.4 - 9.3	<0.01
2008 criteria	**OR**	**95%-CI**	**p value**	**OR**	**95%-CI**	**p value**
Interface hepatitis (≥2)	1.7	0.5 - 5.2	0.4	2.2	0.6 - 8.4	0.2
Rosettes (≥2)	2.9	1.2 - 7.1	0.02	3.5	1.3 - 9.2	0.01
Emperipolesis	2.5	0.9 - 6.8	0.08	3.2	1.0 - 9.8	0.05
1999 and 2008 criteria (fixed model)	**OR**	**95%-CI**	**p value**	**OR**	**95%-CI**	**p value**
Interface hepatitis (≥2)	1.5	0.5 - 4.9	0.5	2.1	0.5 - 8.4	0.3
Plasma cells (≥2)	1.4	0.5 - 3.6	0.5	1.2	0.4 - 3.4	0.8
Rosettes (≥2)	2.9	1.2 - 7.0	0.02	3.5	1.3 - 9.1	0.01
Emperipolesis	2.2	0.8 - 6.4	0.13	3.0	0.9 - 9.8	0.07
1999 and 2008 criteria (stepwise selection)	**OR**	**95%-CI**	**p value**	**OR**	**95%-CI**	**p value**
Rosettes (≥2)	3.0	1.2 - 7.2	0.02	3.6	1.4 - 9.5	<0.01
Emperipolesis	3.0	1.2 - 7.5	0.02	4.0	1.4 - 11.7	0.01

Logistic regression was performed in autoimmune hepatitis (AIH) cases (n=45) and chronic viral hepatitis (n = 56) with all features available. Separate analysis of cases with a definite diagnosis of AIH (pre-treatment IAIHG-score of ≥15, n=34) was also performed. Multivariate logistic regression analysis of the combined 1999 and 2008 features is depicted before (fixed) and after conditional stepwise regression. IAIHG-score, International AIH group diagnostic score.

DISCUSSION

In this study we used a standardized, descriptive method for the assessment of the typical histological features described in the 1999 and 2008 AIH criteria. Literature so far lacks a clear definition or cut-off value to assess the presence of emperipolesis in

the diagnosis of AIH, despite being reported in up to 75-80% of AIH biopsies in previous studies.[4, 13-15] The scoring strategy used in this study to ascertain the presence of emperipolesis showed a high sensitivity for AIH. Also, the use of reticulin staining and semi-quantitative assessment of rosette formation also proved to be a sensitive method. We found a statistically significant difference in the frequencies of all typical AIH features between AIH and chronic viral hepatitis biopsies. However, emperipolesis and rosettes proved to be the only significant independent predictors of AIH in this adult population of chronic hepatitis patients in a multivariate logistic regression analysis. Our results validate and further support the implementation of emperipolesis in favour of plasmacellular dominated infiltrates (1999 criteria) as a separate typical feature in the 2008 simplified scoring system in adults.[4, 14] This may be different in younger patients, as Kumari et al. (2013) recently showed that rosette formation was the only independent histological predictor of AIH (sensitivity 68%, specificity 98%) in children.[13]

A recent study by Rubio et al. (2013), reported that multiple myeloma-1 (MUM-1) staining has potential as an accurate visualisation method for the number and distribution pattern of plasma cells in a small cohort of AIH patients.[16] Due to the retrospective nature and absence of available material, we did not perform MUM-1 staining in this study. Yet, as the recognition of plasma cells can be challenging in H&E stains, future studies should aim to ascertain the potential additional value of MUM-1 staining in the diagnosis and prognosis of AIH. The finding of histological biliary damage in AIH has been reported by Bach (1992) and Czaja (2001) in up to 24-60% of well-defined AIH patients, with destructive cholangitis being present in 3-7%.[8, 17] Similarly, the majority of AIH patients in our study displayed biliary damage and over one fourth had moderate to severe lymphocytic cholangitis. We show that a three point deduction for biliary changes in the 1999 diagnostic score affect the sensitivity to make a definite diagnosis of AIH. In the absence of other clinical or histological features that are typical for alternative or overlapping diagnoses with PBC or PSC, biliary inflammation may therefore be considered compatible with, instead of atypical for AIH.[2, 18, 19]

It has been proposed that the high sensitivity of the 1999 criteria and the high specificity of the 2008 criteria are attributes that can be exploited in the appropriate clinical setting.[4-7] Our results suggest that a future diagnostic model can benefit from an adjusted histological score using the here proposed semi-quantitative assessment methods for emperipolesis, rosettes and biliary inflammation. Portal and lobular histological inflammation were more prominent in AIH than in chronic viral hepatitis, which was reflected in significant differences in ALT and AST levels between the two groups. We refrained from staging biopsies in cases that displayed parenchymal collapse due to bridging necrosis (AIH: 40%) to prevent false positive scores for cirrhosis. This may explain the low frequency of cirrhotic AIH patients found in this study, whereas previous studies reported considerably varying frequencies from 25% to 90%.[8, 9, 20]

Liver histology examination can be limited by sampling error and intra-observer variation, but large inconsistencies in standardized, semi-quantitative scores have been reported to be low if the assessment is performed by an expert hepatopathologist.[21]

In conclusion, we describe a practical, descriptive method to ascertain the presence of emperipolesis and rosettes as diagnostic hallmarks of AIH. These features appear to be superior to interface hepatitis and plasma cell rich infiltrates as histological predictors of AIH in chronic hepatitis and thus may help to establish a clinical diagnosis of AIH. In addition, the presence of moderate to severe lymphocytic cholangitis does not preclude the diagnosis of AIH as it may be present in over one-fourth of AIH liver biopsies.

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